<Original Articles>Mitochondrial Changes in Phospholipid Molecular Species during the Increased Oxidative Phosphorylation after Hepatectomy

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Mitochondrial Changes in Phospholipid Molecular Species during the Increased Oxidative Phosphorylation after Hepatectomy[1]

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Abstract

The changes in liver mitochondrial and microsomal phospholipid molecular species were analyzed during the period of remarkably increased oxidative phosphorylation following partial hepatectomy in rabbits.

At 24 hours after hepatectomy, phosphorylative activity increased significantly from 69.7±5.5 to 118.5±5.7 nmol of ATP synthesized/min/mg protein, compared to the sham operated group.

The ratio of phosphatidylethanolamine to phosphatidylcholine (PE/PC) in mitochondria increased significantly in the hepatectomy group compared with the sham operated group. Remarkable changes in molecular species were observed in mitochondrial phosphatidylethanolamine. 1-Stearoyl-2-arachidonoyl species decreased in the hepatectomy group. On the other hand, microsomal phospholipids hardly changed compared with mitochondrial ones. The change in content of 1-stearoyl-2-arachidonyl phosphatidylethanolamine in mitochondria tended to return to normal levels concomitant with the normalization of phosphorylative activity.

The changes in content of mitochondrial phospholipids, especially phosphatidylethanolamine, might also be related to enhancement of phosphorylative activity.

Key words: Hepatectomy, Mitochondria, Phosphorylative activity, Phospholipid, Molecular species.
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索引語：肝切除術，ミトコンドリア，リン酸化能，リン脂質，分子種.

Abbreviations: CL, cardiolipin; PE, phosphatidylethanolamine; PC, phosphatidylcholine; GC-MS, gas chromatography-mass spectrometry; $PE_{38:4}$, phosphatidylethanolamine with numbers indicating sums of the chain length and of the unsaturation of fatty acids esterified at positions C-1 and C-2 of glycerol; HPLC, high performance liquid chromatography; *t*BDMS, *tert*-butyldimethylsilyl; DNB, dinitrobenzoyl; EI, electron impact.

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It is well recognized that phospholipids can regulate the activity of membrane-bound enzymes and membrane transport in a manner dependent on their polar head group as well as their fatty acid composition[1]. The electron transport chain in the membrane of mitochondria is buried in a milieu of phospholipids, and the dependence of membrane bound enzymes on phospholipids is well documented[2,3,4]. In fact, mitochondrial function is affected by the modification of membrane phospholipids with dietary lipids[5].

Recently, we found that a decrease in cardiolipin content and changes in its fatty acid composition occurred in the mitochondria of liver whose function had been severely impaired due to orthotopic liver transplantation. Cyclosporine had the effect of protecting the liver from phospholipid changes and preventing the deterioration of mitochondrial function, indicating that the deterioration of mitochondrial function upon acute rejection might be due to the changes in cardiolipin[6].

In marked contrast to the rejection model, mitochondrial oxidative phosphorylation increases to nearly twice the normal level at 24 hours after partial hepatectomy in rabbits. This highly increased mitochondrial oxidative phosphorylative level reflects a compensatory mechanism to overcome the decreased energy charge due to metabolic overload on the liver. The increased phosphorylative activity returns to normal level at 7 days after hepatectomy along with the normalization of energy charge.

In this context, we first analyzed mitochondrial phospholipids, focussed on 24 hours after hepatectomy. Then, we investigated a time course of change in mitochondrial phospholipid to clarify the relationship between change in phospholipid and the mitochondrial function.

Materials and Methods

Materials

Phospholipase C (EC 3.1.4.2) was prepared from the culture medium of *Bacillus cereus* as previously described[7]. 1,2-Dilauroyl, 1-palmitoyl-2-oleoyl, 1-palmitoyl-2-linoleoyl, 1-stearoyl-2-oleoyl, 1-stearoyl-2-linoleoyl, 1-stearoyl-2-arachidonoyl, 1,2-dioleoyl and 1-oleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholines were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Linolenic acid was purchased from Nu-Check Prep Inc. (Elysian, MN, USA). 1-Palmitoyl-2-linolenoyl- and 1-srearoyl-2-linolenoyl-*sn*-glycero-3-phosphocholines were synthesized as previously described[7]. 3,5-Dinitrobenzoyl chloride (DNBC) was from Dojindo Laboratories (Kumamoto, Japan). Acetonitrile and 2-propanol were HPLC grade. All other chemicals were of reagent grade.

Animals

Healthy young male rabbits, weighing 2.0–2.5 kg, were used in this study. The animals were maintained on laboratory food (Clea-1, Nippon Clea Co. Ltd.) and water *ad libitum* for 2 weeks before operation. No food was given from 12 hours before to 24 hours after operation. The 70% hepatectomized rabbits had the left anterior, right anterior, and right posterior lobes resected under anesthesia induced by *i.v.* injection of sodium 5-allyl-5-(1-methyl)-2-thiobarbiturate at 15 mg per kg of body weight[8]. Sham operation was performed by dissecting the hepatic ligaments and palpating the liver gently.

Preparation of mitochondria and microsomes

The liver mitochondria were prepared according to the method of Ozawa *et al.* using 0.3 M manitol (Mituwa Pure Chemical, Japan)/0.1 mM EDTA (pH 7.4) as a isolation medium[9]. Measurement of mitochondrial phosphorylative activity was performed using a Hagiwara type

polarography as previously described (10). For separation of microsomal fraction, the postmitochondrial supernatant was centrifuged first at 15,000 g for 15 min. The sediment was discarded, and microsomal fraction was obtained from the supernatant by centrifugation at 105,000 g for 60 min.

Extraction and separation of phospholipids

Total lipids were extracted from the mitochondrial and microsomal suspensions by the modified method of BLIGH and DYER[11], as was described by ISHINAGA *et al.*[12]. Total lipids in chloroform and methanol (2 : 1, v/v) were applied to a preparative Silica gel 60 plate (E. Merck, Darmstadt, FRG). Phospholipids were separated by two-dimensional thin layer chromatography following the method of ROUSER *et al.*[13]. Phospholipids were detected by their fluorescence under ultraviolet light after spraying the plate with 0.03% 2-*p*-toluidynylnaphtarene-6-sulfonic acid (Nakarai Chemicals, Kyoto, Japan). Phospholipids were removed by scraping and extracted three times by the method of BLIGH and DYER[11]. Phospholipid content was calculated from the amount of lipid phosphorus determined by the method of BARTLETT[14]. Protein was determined by the method of LOWRY *et al.*[15], using bovine serum albumin (Sigma, St. Louis, USA) as a standard.

Measurement of fatty acid composition

Phospholipids were refluxed with 10% methanolic HCl (Tokyo Kasei, Tokyo, Japan) for 1 hour. The resulting fatty acid methyl esters were extracted with *n*-hexane followed by washing with water. A Shimadzu GC-7A apparatus with a flame ionization detector was used. The column was a glass tube (2 m × 3 mm) packed with 10% Silar 10-C on Gas Chrome-Q (100–120-mesh). The column temperature was kept at 185°C for 8 min, increased to 215°C at a rate 5°C/min, and then maintained at this temperature. Peak areas were calculated automatically with a Shimadzu C-RIA Chromatopac computer.

Preparation of diacylglycerol and their derivatization

The phospholipid to be analyzed (5 μg of phosphorus) and 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (0.5 μg of phosphorus) were hydrolyzed with phospholipase C as previously described[7]. The resulting diacylglycerol was converted either to dinitrobenzoyl (DNB) derivatives according to the method of KITO *et al.*[16] or to *t*BDMS derivatives by the method previously described[7].

Separation of molecular species by high performance liquid chromatography (HPLC)

HPLC was carried out on a TOYO SODA CCPM dual pump system. A type ODS-120-T column packed with TSK gel was used for separation of molecular species. The solvent system was acetonitrile/2-propanol (4 : 1, v/v) and the flow rate was 0.75 ml/min. The separated molecular species were detected by measuring the absorption at 254 nm with a TOYO SODA UV-8000 apparatus. Peak areas were calculated with Shimadzu model C-R5A Chromatopac.

The relative retention times of DNB derivatives of samples to dilauroyl one were compared by HPLC runs of each DNB derivative made from standard phospholipid and dilauroyl PC. PC from human platelets were also used as a standard for the polyunsaturated species[17].

Graphical representation of the relative retention time values for the molecular species was then carried out according to NAKAGAWA and HORROCKS[18] and PATTON *et al.*[19].

Mass chromatography

*t*BDMS derivatives of molecular species were analyzed by gas chromatography mass-spectrometry (GC-MS) as previously described[20] to re-evaluate the results by HPLC. The apparatus employed was a JEOL JMS-DX 300 equipped with a JMA-3000 data system. The column was a 1 m × 2 mm glass spiral packed with 1% OV-1 on Chromosorb W (80–100 mesh). The temperatures of the column, injection port, separator, inlet, and ionization chamber were maintained at 290, 290,

290, 290, and 265°C, respectively. The flow rate of helium gas was kept at 52.5 ml/min. The electron energy was 70 eV. Mass chromatography was performed from repetitive scans obtained at 6 sec intervals. The ion at $[M-57]^+$ was chosen as being diagnostic of molecular weight. The areas monitored at $[M-57]^+$ were integrated.

Statistical analysis

Statisyical analysis of data was performed with STUDENT's t-test between the sham and hepatectomy groups. The value less than 0.01 was determined to be significant.

Results

A. *Result at 24 hours after hepatectomy*

Mitochondrial phosphorylative activity

The mitochondrial phosphorylative activity was 118.5±5.7 nmol (n=7, mean±SEM) of ATP synthesized per min per mg protein in the remnant liver from the hepatectomy group and 69.7±5.5 nmol (n=5, mean±SEM) in the sham operated group. The respiratory control ratio was 8.0±0.4 in the former and 4.7±0.3 in the latter.

Phospholipid content

The phospholipid : protein ratio in mitochondria was 173.9±6.0 (mean±SEM) nmol per mg protein in the hepatectomy group and 173.0±6.6 in sham operated group. In microsomes, it was 806.5±20.4 and 741.9±22.3, respectively. These values did not differ significantly from each other.

Phospholipid contents of liver mitochondria and microsomes are shown in Table 1.

Phospholipid contents of liver mitochondria and microsomes in the sham group were almost identical with the results obtained by other laboratories[21,22].

There was no significant difference in microsomes between the sham and the hepatectomy groups. In contrast, alteration in phospholipid content due to hepatectomy was found in mitochondria, namely, the ratio of PE to PC significantly increased in the hepatectomy group. There was no

Table 1 Phospholipid compositions of mitochondria and microsomes (%)

Phospholipid	Mitochondria		Microsomes	
	Sham (n=5)	Hepatectomy (n=7)	Sham (n=4)	Hepatectomy (n=4)
CL	12.3±0.5	12.4±0.5	0.9±0.1	0.6±0.1
PE	40.2±1.3	42.6±0.6	25.5±1.6	27.6±0.4
PC	40.3±0.8	37.0±0.8	58.8±0.7	59.8±0.6
PS[a]	3.7±0.6	3.6±0.4	8.5±0.6	7.8±0.5
PI[b]	2.1±1.0	2.1±0.8	0.6±0.1	0.5±0.2
SM[c]	0.8±0.1	1.2±0.4	4.1±0.6	2.7±0.3
LPC[d]	0.5±0.2	1.0±0.3	1.5±0.4	0.7±0.1
PG[e]	N.D.	N.D.	0.2±0.1	0.3±0.1
PE/PC[f]	1.00±0.10	1.16±0.06*	0.44±0.03	0.46±0.01

Results are expressed as mean±SEM. Experimental numbers are shown in the parenthesis.

* <0.01 as calculated by STUDENT t-test between sham and hepatectomy groups.

N.D., not detected. [a], phosphatidylserine; [b], phosphatidylinositol; [c], sphingomyelin; [d], lyso-phosphatidylcholine; [e], phosphatidylglycerol; [f], the ratio of phosphatidyl ethanolamine to phosphatidylcholine.

Table 2 Fatty acid compositions of PC in mitochondria and microsomes (%)

Fatty acid	Mitochondria		Microsomes	
	Sham (n=5)	Hepatectomy (n=7)	Sham (n=4)	Hepatectomy (n=4)
C 16 : 0	22.5±0.8	22.4±0.8	21.1±0.7	22.2±0.9
C 16 : 1	0.8±0.2	1.8±0.2	1.3±0.1	1.4±0.1
C 18 : 0	14.1±0.3	12.7±0.4	17.6±0.5	15.3±0.8
C 18 : 1	6.8±0.9	10.4±0.9	8.2±1.6	9.2±0.5
C 18 : 2	29.9±1.1	30.3±0.5	30.4±0.6	31.1±0.5
C 18 : 3	4.2±0.4	4.7±0.2	3.0±0.2	3.7±0.3
C 20 : 3	2.3±0.6	1.6±0.6	2.6±0.6	2.3±0.2
C 20 : 4	6.1±0.6	5.0±0.3	5.0±0.1	3.7±0.3
C 20 : 5	2.3±0.3	1 8±0.2	1.3±0.2	1.0±0.3
C 22 : 5	1.2±0.2	1.2±0.2	0.9±0.2	0.7±0.2
C 22 : 6	4.1±0.4	3.4±0.5	5.7±1.0	5.6±0.3

Results are expressed as in the legend to Table 1.

significant change in content of cardiolipin, a marker lipid for mitochondria.

Fatty acid composition

Fatty acid composition of cardiolipin in mitochondria did not show a significant change between sham and hepatectomy groups (Data is not shown). Fatty acid compositions of mitochondrial and microsomal PC did not show a difference between sham and hepatectomy groups (Table 2).

Phosphatidylethanolamine (PE) showed significant differences between sham and hepatectomy groups as well as the decided difference between mitochondria and microsomes (Table 3). Mitochondrial PE contained more C 20 : 4, compared with the microsomal one. Significant alteration due to hepatectomy was seen in fatty acid composition of mitochondrial PE. C 20 : 4 decreased significantly, while C 18 : 1 and C 18 : 3 showed inverse increases in the hepatectomy group. Contrary to mitochondria, microsome did not show a significant change.

Molecular species of phosphatidylcholine

Table 3 Fatty acid composition of PE in mitochondria and microsomes (%)

Fatty acid	Mitochondria		Microsomes	
	Sham (n=5)	Hepatectomy (n=7)	Sham (n=4)	Hepatectomy (n=4)
C 16 : 0	11.8±0.2	11.4±0.4	11.4±0.5	12.9±0.6
C 16 : 1	1.0±0.1	1.5±0.1	1.9±0.2	1.5±0.2
C 18 : 0	26.1±0.4	24.4±0.7	26.7±1.1	23.4±0.7
C 18 : 1	5.0±0.6	8.5±0.6*	7.9±1.2	9.7±0.2
C 18 : 2	17.2±1.0	19.6±0.6	22.9±0.5	23.2±0.6
C 18 : 3	2.8±0.4	4.2±0.2*	3.6±0.3	4.5±0.5
C 20 : 3	1.3±0.1	2.2±0.5	1.9±0.2	1.9±0.4
C 20 : 4	18.6±1.1	14.2±0.8*	10.8±0.5	8.7±0.5
C 20 : 5	1.8±0.6	1.6±0.2	1.4±0.7	2.0±0.7
C 22 : 5	2.8±0.2	2.4±0.1	1.5±0.4	1.3±0.5
C 22 : 6	5.9±0.7	4.8±0.3	6.5±0.6	8.1±0.6

*, $p<0.01$ compared with sham group by STUDENT t-test.

Table 4 Molecular species of PC in mitochondria and microsomes (%)

Peak number	Molecular species	Mitochondria		Microsomes	
		Sham (n=5)	Hepatectomy (n=7)	Sham (n=4)	Hepatectomy (n=4)
1	18 : 1/18 : 3	3.8±0.3	4.6±0.3	3.3±0.5	4.2±0.2
2	16 : 0/22 : 6	1.4±0.1	1.7±0.1	1.6±0.5	1.1±0.0
3	18 : 1/20 : 4 +16 : 0/18 : 3	4.1±0.5	4.9±0.2	2.7±0.4	3.5±0.5
4	16 : 0/22 : 5	1.2±0.1	1.0±0.1	0.7±0.2	0.5±0.1
5	16 : 0/20 : 4	4.7±0.5	3.8±0.2	3.0±0.2	2.6±0.2
6	18 : 1/18 : 2	2.9±1.1	4.4±1.1	2.0±0.6	1.5±0.4
7	18 : 0/22 : 6	2.7±0.2	4.6±0.1*	2.8±0.5	3.7±0.4
8	16 : 0/18 : 2				
	16 : 0/16 : 1	37.6±1.8	31.9±1.5	33.3±1.8	36.7±0.6
	18 : 1/22 : 4				
9	18 : 0/18 : 3	2.4±0.3	2.6±0.1	2.6±0.4	2.2±0.4
10	18 : 1/20 : 4	4.6±0.8	3.4±0.3	4.0±0.4	2.9±0.2
11	18 : 1/18 : 1	2.4±1.2	3.5±0.3	1.9±0.3	1.1±0.5
12	18 : 0/18 : 2	29.6±1.4	28.1±1.9	32.5±1.3	33.1±0.4
	16 : 0/18 : 1				
13	16 : 0/16 : 0	0.5±0.1	0.6±0.1	N.D.	N.D.
14	18 : 0/18 : 1	2.1±0.7	3.4±0.7	3.8±0.7	3.3±0.4

Results were expressed as the legend to Table 1. Peak numbers correspond to the number of peaks in Fig. 1.

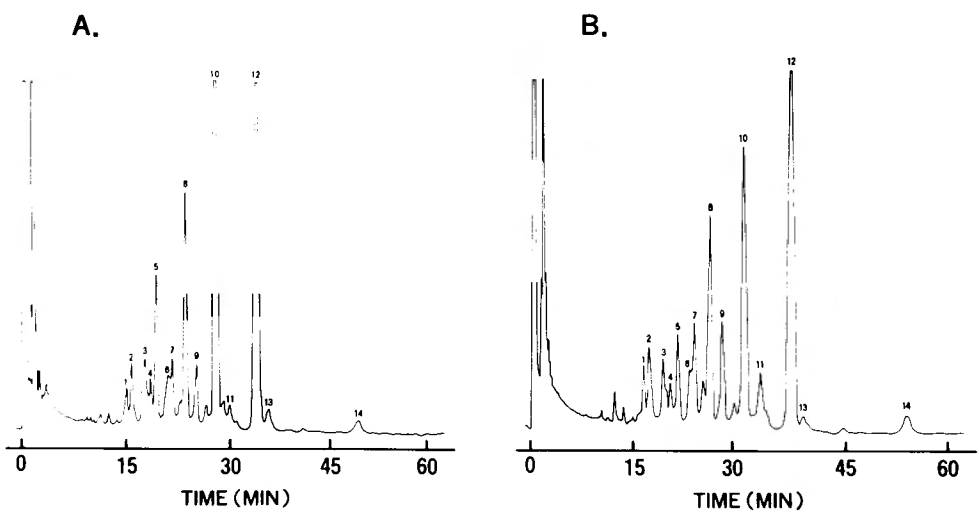

Fig. 1 HPLC separation of molecular species of DNB derivatives prepared from mitochondrial PE: A, in sham group; B, in hepatectomy group. DNB derivatives suitable to 5 μg of lipid phosphorus were dissolved in 500 μl of acetonitrile, 10 μl of sample was injected, and chromatographed on ODS-120-T column. The solvent mixture was acetonitrile/2-propanol (4 : 1, v/v) and flow rate was 0.75 ml/min. Detection was by absorption at 254 nm. The peak numbers correspond to those in Table 4. Fourteen peaks were identified in these samples. They were expressed as the combination of fatty acids esterified at C-1 and C-2 positions of glycerols, i.e., 18 : 0/18 : 2.

Phosphatidylcholine separated into 14 molecular species by HPLC. They were identified by the fatty acids at the position of C-1 and C-2 of glycerol.

The molecular species in the microsomes were nearly the same as in mitochondria and were not changed significantly by hepatectomy (Table 4).

The major molecular species in mitochondria were 16 : 0/18 : 2, 16 : 0/18 : 1, and 18 : 0/18 : 2. Changes were seen in 18 : 0/22 : 6 species from mitochondria between the sham and the hepatectomy groups.

Molecular species of phosphatidylethanolamine

HPLC profile of mitochondrial PE is shown in Fig. 1. The major molecular species in the microsomes were 18 : 0/18 : 2, 16 : 0/18 : 1, 16 : 0/18 : 2, 18 : 0/20 : 4, and 18 : 0/22 : 6. A striking similarity was found between the sham and hepatectomy group in microsomes.

The major molecular species in mitochondria were 18 : 0/20 : 4, 18 : 0/18 : 2, 16 : 0/18 : 2, 16 : 0/20 : 4. Marked change due to hepatectomy was observed, especially in 18 : 0/20 : 4 which decreased remarkably in the mitochondria of the hepatectomy group. This decrease in molecular species containing arachidonic acid was compensated for by the increase in 18 : 1/18 : 1 and 18 : 0/18 : 3 species (Table 5).

B. *Time course of the relative intensity of $PE_{38:4}$ with respect to $PE_{36:2}$ in mitochondria by gas-chromatography mass spectrometry*

To know if the change in content of 18 : 0/20 : 4 PE is related to phosphorylative activity, the relative intensity of $PE_{38:4}$ with respect to $PE_{36:2}$, major species in mitochondria, and phosphorylative activity were measured at 12, 96 hours and on 7 days after hepatectomy. The ratio of the value in the hepatectomy group to that in sham group was calculated, respectively (Table 6).

Table 5 Molecular species of PE in mitochondria and microsomes (%)

Molecular species	Mitochondria		Microsomes	
	Sham (n=5)	Hepatectomy (n=7)	Sham (n=4)	Hepatectomy (n=4)
18 : 1/18 : 3	2.3±0.7	2.6±0.3	3.0±0.3	3.3±0.5
16 : 0/22 : 6	4.2±0.5	3.6±0.4	4.2±0.5	4.2±0.6
18 : 1/20 : 4 16 : 0/18 : 3	3.5±0.3	4.3±0.2	3.2±0.4	3.8±0.6
16 : 0/22 : 5	2.3±0.3	2.0±0.1	1.9±0.3	2.4±0.7
16 : 0/20 : 4	6.2±0.4	4.8±0.3	3.5±0.2	3.0±0.2
18 : 1/18 : 2	3.1±0.4	4.0±0.8	1.4±0.3	1.1±0.1
18 : 0/22 : 6	3.2±0.6	5.0±0.5	5.5±0.8	7.3±0.8
16 : 0/18 : 2 16 : 0/16 : 1	11.6±0.7	10.5±0.6	15.0±1.0	17.0±0.9
18 : 0/18 : 3	4.7±0.7	7.4±1.0*	3.6±0.1	4.0±0.6
18 : 0/20 : 4	23.7±1.4	15.2±1.0*	13.2±0.6	10.9±0.5
18 : 1/18 : 1	2.4±0.5	6.4±1.5*	1.6±0.2	2.0±0.2
18 : 0/18 : 2 16 : 0/18 : 1	24.2±0.9	22.9±1.8	31.7±0.5	33.0±0.9
16 : 0/16 : 0	1.6±0.1	1.1±0.1	N.D.	N.D.
18 : 0/18 : 1	1.2±0.2	2.2±0.3	2.3±0.4	3.4±0.2

Results are expressed as in the legend to Table 1 and 3.

Table 6 Time course of the relative intensity of $PE_{38:4}$ with respect to $PE_{36:2}$ and the phosphorylative activity after hepatectomy

The values are expressed as the ratio of the hepatectomy group to the sham group, respectively. We chose the sham group as a control to the hepatectomy group. Accordingly, the ratios of the values of the hepatectomy group to the sham group were expressed. Respective values of $PE_{38:4}/PE_{36:2}$ and phosphorylative activity are as the followings: $PE_{38:4}/PE_{36:2}$; 12 hours, sham group 4.97±1.21 (n=3) and hepatectomy group 2.37±0.0.49 (n=3), 24 hours, 4.34±0.84 (n=5) and 1.83±0.24 (n=7), 96 hours, 2.87±0.23 (n=4) and 2.15±0.30 (n=4), 7 days, 2.99±0.54 (n=4) and 2.88±0.67 (n=4); Phosphorylative activity (nmol ATP synthesized per min. per mg protein), 12 hours, 54.33±3.93 and 88.89±10.93, 24 hours, 69.70±5.51 and 118.49±5.73, 96 hours, 53.36±0.93 and 71.23±5.44, 7 days, 56.58±6.30 and 68.78±6.30. These values were expressed as mean±SEM. Experimental number is in the parenthesis.

	12 hrs	24 hrs	96 hrs	7 days
$PE_{38:4}/PE_{36:2}$	0.48	0.42	0.75	0.96
P R[a]	1.62	1.70	1.33	1.21

[a] phosphorylation rate.

The ratio of $PE_{38:4}$ to $PE_{36:2}$ decreased as phosphorylative activity increased, and showed the lowest value at 24 hours after hepatectomy when phosphorylative activity showed the highest value. The change in phospholipid tended to return to normal level concomitant with the normalization of phosphorylative activity.

Discussion

Naturally occurring phospholipids are composed of many molecular species, whose composition can be altered dramatically in response to physiological and pathological factors[23]. Many studies on subcellular membrane phospholipids in the regenerating liver have been done, and it is commonly accepted that the mitochondrial membrane phospholipid composition changes only slightly during regeneration[24]. However, there are no studies on molecular species of the mitochondrial membrane phospholipids at the period of increased oxidative phosphorylation after partial hepatectomy.

The aim of the present study is to investigate the changes in mitochondrial membrane phospholipids at the level of molecular species during the period of remarkably increased oxidative phosphorylation.

Although it is commonly thought that cardiolipin plays a most important role on mitochondrial function, a significant difference was observed neither in the content of cardiolipin (Table 1) nor in their fatty acid composition in our case.

In mitichondria, the ratio of PE to PC significantly increased at 24 hours after hepatectomy. However, the phospholipid composition in microsome showed no change by hepatectomy. KAGAWA and RACKER showed that phosphorylative activity changed by phospholipid composition in reconstituted vesicles[3].

There was not a significant difference in the composition of fatty acid and molecular species in PC between the mitochondria and microsomes (Table 2, 4).

The changes in molecular species occurred in the mitochondrial PE. 1-Stearoyl-2-arachidonyl PE, an important precursor and reservoir of bioactive eicosanoid, decreased remarkably by hepatectomy. This species changed in inverse proportion to phosphorylative activity (Table 6). Accordingly, it is suggested that phosphorylative activity might be related to the change in 1-stearoyl-2-arachidonoyl PE. Arachidonic acid is released from membrane phospholipids by phospholipase

A_2[25]. De Winter *et al.* and Lenting *et al.* reported that mitochondrial phospholipase A_2 hydrolyzed PE preferentially[26,27]. Although parameters potentially important for determining rate of ATP synthesis are complex and have not been determined, the phospholipid changes observed in mitochondrial PE may be one of the potential for ATP synthesis.

Recently, Aldail *et al.* reported that there are differences in lipid compositions of outer membrane, inner membrane and each membrane's contact sites and that their membrane fractions have different physicochemical properties[28]. Accordingly, further purification of mitochondrial membrane components may be needed in our study to get clearer relationship between changes in phospholipid composition and the phosphorylative activity of mitochondria.

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和文抄録

肝切除後の酸化的リン酸化能の亢進時における ミトコンドリア膜リン脂質分子種の変化

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ウサギの70%肝切除後，残存肝のエネルギー需要の増大のため energy charge は低下する．ミトコンドリアは低下した energy charge を回復するため，その酸化的リン酸化能が著明に亢進することが知られている．一方，膜リン脂質の組成が膜機能を変化させることも知られている．今回，肝切除後のミトコンドリアの酸化的リン酸化能の亢進と膜リン脂質の関連性をあきらかにするため，酸化的リン酸化能とミトコンドリア膜リン脂質の変化を測定した．

肝切除後24時間でミトコンドリアの酸化的リン酸化能は，対照群 69.9±5.5 ATP nmol/min/mg protein に比べて，肝切除群では 118.5±5.7 nmol/min/mg protein に増加した ($p<0.01$)．リン脂質分子種の有意な変化は，ホスファチジルエタノールアミン (PE) にみられ，肝切除群で 1-ステアロイル-2-アラキドニル分子種 (18 : 0/20 : 4 PE) が減少し，1,2-ジオレオイルや 1-ステアロイル-2-リノレノイル分子種 (18 : 1/18 : 1 PE や 18 : 0/18 : 3 PE) が増加していた ($p<0.01$)．これに対し，マイクロゾームのリン脂質は肝切除によって全く変化しなかった．このミトコンドリアにおける PE 分子種の変化は，肝切除後の酸化的リン酸化能の正常化とともに対照群値に戻った．

ミトコンドリア膜でのリン脂質分子種の変化が肝切除後の酸化的リン酸化能の増加に関連している可能性が示された．